

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Original) An isolated culture of *Chrysosporium lucknowense* Garg 27K having accession number VKM F-3500D.
- 2-5. (Cancelled)
6. (Previously presented) A composition according to claim 112, wherein the fungus is a mutant strain of *Chrysosporium lucknowense* Garg 27K.
7. (Previously presented) A composition according to claim 112 having cellulase activity at a temperature from about 40°C to about 60°C, at a pH from about 5.0 to about 11.0.
8. (Previously presented) A composition according to claim 112 having at least 50% of the optimal cellulase activity, at a pH from about 6.0 to about 7.0, at a temperature from about 40°C to about 60°C.
9. (Previously presented) A composition according to claim 112 wherein said cellulase activity is assayed by any one of the CMCase, RBBCMCase, endoviscometric or filter paper activity assays.
10. (Currently amended) A substantially purified and isolated protein fraction, obtained from a composition according to claim 112, and having at least 50% of its maximal cellulase activity at a pH between about 6.0 and about 7.0 as ~~measured by any one of the CMCase, RBBCMCase, endoviscometric or filter paper activity assays.~~

11. (Original) An endoglucanase obtained from a fraction according to claim 10, having a molecular weight of about 25 kD and pI of about 4.
12. (Original) An endoglucanase obtained from a fraction according to claim 10, having a molecular weight of about 70 kD and a pI of about 4.
13. (Original) An endoglucanase obtained from a fraction according to claim 10, having a molecular weight of about 60 kD and a pI of about 3.
14. (Original) An endoglucanase obtained from a fraction according to claim 10, having a molecular weight of about 43 kD and a pI of about 3.
15. (Original) A cellobiohydrolase obtained from a fraction according to claim 10, having a molecular weight of about 60 kD and a pI of about 4.
16. (Original) A substantially purified and isolated neutral and/or alkaline cellulase enzyme, isolated from a protein fraction according to claim 10, and having a pI of between about 3 and about 5.5.
17. (Original) A cellulase according to claim 16 wherein said cellulase possesses either endoglucanase or cellobiohydrolase activity.
18. (Original) A cellulase according to claim 16 wherein said cellulase retains at least 50% of its maximal cellulase activity at a pH between about 6.0 and about 7.0.
19. (Original) An endoglucanase obtained from a fraction according to claim 10 and having a molecular weight of about 25 kD.
20. (Original) An endoglucanase obtained from a fraction according to claim 10 and having a molecular weight of about 70 kD.
21. (Original) An endoglucanase obtained from a fraction according to claim 10 and having a molecular weight of about 43 kD.
22. (Original) A detergent composition containing one or more purified enzymes isolated from a protein fraction according to claim 10, and further comprising a surfactant.

23. (Original) A fabric softening composition containing one or more purified enzymes obtained from the protein fraction according to claim 10.
24. (Currently amended) A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, comprising an isolated cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*, wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D, said composition having a pH between about 8.0 and about 12.0.
- 25-27. (Cancelled)
28. (Currently amended) A composition according to ~~any one of claims claim~~ 24 24-26, further comprising one or more components selected from the group consisting of pumice stones, abrasives, softeners, solvents, preservatives, bleaching agents, bluing agents, fluorescent dyes, antioxidants, solubilizers, detergents, surfactants, enzymes, builders, anti-redeposition agents, buffers, caking inhibitors, masking agents for factors inhibiting the cellulase activity, and cellulase activators.
29. (Cancelled)
30. (Original) The composition of claim 24, wherein the pH is between 10.0 and about 11.0.
- 31-33. (Cancelled)
34. (Original) The composition of claim 28, wherein the pH is between 10.0 and about 11.0.
35. (Cancelled)
36. (Currently amended) A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, comprising an isolated cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*, wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D, said

composition further comprising one or more components selected from the group consisting of proteinases, detergents, and surfactants, ~~said composition has cellulase activity at a temperature between 40 °C and 60 °C, and at a pH of about 5 to about 12.~~

37-39. (Cancelled)

40. (Currently amended) A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least ~~424~~ about 33 units of ~~endo-1,4-(3-endo-1,4-β-glucanase~~ activity per gram of dry composition, as measured by an endovisco-metric assay, of an isolated cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*, wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D, ~~said composition has cellulase activity at a temperature between 40 °C and 60 °C, and at a pH of about 5 to about 12.~~

41-42. (Cancelled)

43. (Currently amended) A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least ~~424~~ about 33 units of ~~endo-1,4-3-endo-1,4-β-glucanase~~ activity per gram of dry composition, as measured by an endovisco-metric assay, of an isolated cellulase obtained from a wild-type or mutant fungus of the genus *Chrysosporium*, wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D, ~~said composition has cellulase activity at a temperature between 40 °C and 60 °C, and at a pH of about 5 to about 12.~~

44-45. (Cancelled)

46. (Currently amended) A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least about 120 ~~494~~ units of ~~endo-1,4-3-endo-1,4-β-glucanase~~ activity per gram of dry composition, as measured by an endovisco-metric assay, of an isolated cellulase whose amino acid

sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*, wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D, said composition has cellulase activity at a temperature between 40 °C and 60 °C, and at a pH of about 5 to about 12.

47-48. (Cancelled)

49. (Currently amended) A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least about 120 ~~194~~ units of ~~endo-1,4-3~~ endo-1,4-β-glucanase activity per gram of dry composition, as measured by an endoviscometric assay, of an isolated cellulase obtained from a wild-type or mutant fungus of the genus *Chrysosporium*, wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D, said composition has cellulase activity at a temperature between 40 °C and 60 °C, and at a pH of about 5 to about 12.

50-51. (Cancelled)

52. (Currently amended) A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least about 964 units of ~~endo-1,4-~~ ~~(3-endo-1,4-β~~ glucanase activity per gram of dry composition, as measured by an endoviscometric assay, of an isolated cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*, wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D, said composition has cellulase activity at a temperature between 40 °C and 60 °C, and at a pH of about 5 to about 12.

53-54. (Cancelled)

55. (Currently amended) A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least about 964 units of endo-1,4-β-glucanase activity per gram of dry composition, ~~as measured by an endoviscometric assay~~, of an isolated cellulase obtained from a wild-type or

mutant fungus of the genus *Chrysosporium*, wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D, said composition has cellulase activity at a temperature between 40 °C and 60 °C, and at a pH of about 5 to about 12.

56-57. (Cancelled)

58. (Currently amended) A laundry detergent composition, comprising an isolated cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*, wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D, further comprising one or more surfactants, said composition has cellulase activity at a temperature between 40 °C and 60 °C, and at a pH of about 5 to about 12.

59-60. (Cancelled)

61. (Currently amended) A laundry detergent composition, comprising an isolated cellulase obtained from a wild-type or mutant fungus of the genus *Chrysosporium*, wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D, further comprising one or more surfactants, said composition has cellulase activity at a temperature between 40 °C and 60 °C, and at a pH of about 5 to about 12.

62-66. (Cancelled)

67. (Withdrawn- Currently amended) A method for producing the composition according to claim 112 ~~2 or 4~~, said method comprising growing a mutant fungus of the genus *Chrysosporium* in culture in a suitable medium.

68-72. (Cancelled)

73. (Withdrawn- Currently amended) A method of stonewashing denim fabric or denim jeans, said method comprising treating said denim fabric or denim jeans with a composition according to any one of claims ~~4, 5, 6, 10, or 24~~, and 112.

74. (Withdrawn- Currently amended) A method of biopolishing, defibrillating, bleaching, dyeing, or desizing textiles comprising treating said textiles with a composition according to any one of claims 4, ~~5~~, 6, 10, ~~or 24~~, and 112.
75. (Withdrawn- Currently amended) A method of deinking or biobleaching paper or pulp, said method comprising treating said paper or pulp with a composition according to any one of claims 4, ~~5~~, 6, 10, ~~or 24~~, and 112.
76. (Withdrawn- Currently amended) A method for enhancing the softness or feel of cellulose or cotton-containing fabric, comprising treating said fabric with a composition according to any one of claims 4, ~~5~~, 6, 10, ~~or 24~~, and 112.
- 77-79. (Cancelled)
80. (Currently amended) A method for generating mutant strains of the genus *Chrysosporium* which produce enhanced cellulase activity at neutral and/or alkaline pH's, comprising
- (a) mutating spores of a fungus of the genus *Chrysosporium*;
 - (b) culturing the spores from step (a); and
 - (c) screening the cultures from step (b) for enhanced levels of neutral and/or alkaline cellulase activity,
- wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D, said cultures have cellulase activity at a temperature between 40 °C and 60 °C, and at a pH of about 5 to about 12.
81. (Original) The method of claim 80 wherein the mutating step comprises exposing the spores to ultraviolet light or a chemical mutagen.
82. (Original) The method of claim 81 wherein the chemical mutagen is nitrous acid, N-methyl-N'-nitro-N-nitrosoguanidine, or 4-nitroquinolone-N-oxide.
83. (Previously presented) A mutant strain of the genus *Chrysosporium* obtained by the method of any one of claims 80 or 81.

84. (Withdrawn- Currently amended) A method of isolating genes encoding cellulase enzymes of *Chrysosporium* comprising:
- a) isolating a protein from a neutral and/or alkaline cellulase composition produced by a wild type or mutant ~~*Chrysosporium*~~ *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D;
 - b) sequencing all or part of the protein isolated in step (a);
 - c) producing a nucleic acid probe derived from the sequence of step (b);
 - d) screening a wild type or mutant *Chrysosporium* library with the nucleic acid probe of step (c);
 - e) isolating a nucleic acid sequence recognized by the probe; and
 - f) sequencing the nucleic acid sequence isolated in step (e).
85. (Withdrawn) A nucleic acid sequence obtained by the method of claim 84.
86. (Withdrawn) An isolated nucleic acid whose sequence encodes a cellulase enzyme, wherein the cellulase enzyme is isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium*.
87. (Withdrawn) An isolated nucleic acid whose sequence encodes a cellulase according to claim 11.
88. (Withdrawn) A recombinant expression vector comprising a nucleic acid sequence according to any one of claims 85 or 86.
89. (Withdrawn) A host cell containing a recombinant expression vector of claim 88.
90. (Withdrawn) A host cell according to claim 89 wherein the host cell is selected from the group consisting of yeast cells, fungal cells, plant cells, and bacterial cells.

91. (Withdrawn) A host cell according to claim 90 wherein the host cell is a fungal cell selected from the group consisting of *Trichoderma*, *Aspergillus*, *Humicola*, *Penicillium*, *Chrysosporium*, and *Neurospora*.
92. (Withdrawn- Currently amended) A method of culturing a fungus of *Chrysosporium lucknowense* Garg 27K having accession number VKM F-3500D the genus *Chrysosporium*, wherein the fungus is grown in a medium containing inorganic salts, carbon sources, and organic nitrogen sources, at a pH between about 5 and 8.
93. (Withdrawn) A method of culturing a fungus of the genus *Chrysosporium* according to claim 92, wherein the pH is between about 6.5 and 7.5.
94. (Withdrawn) A method of culturing a fungus of the genus *Chrysosporium* according to claim 92, wherein the pH is between about 6.9 and 7.1.
95. (Withdrawn) A method of culturing a fungus of the genus *Chrysosporium* according to claim 92, wherein the pH is maintained at 7.5.
96. (Withdrawn- Currently amended) An isolated nucleic acid whose sequence encodes a cellulase ~~cellulose~~ according to claim 12.
97. (Withdrawn- Currently amended) An isolated nucleic acid whose sequence encodes a cellulase ~~cellulose~~ according to claim 13.
98. (Withdrawn- Currently amended) An isolated nucleic acid whose sequence encodes a cellulase ~~cellulose~~ according to claim 14.
99. (Withdrawn- Currently amended) An isolated nucleic acid whose sequence encodes a cellulase ~~cellulose~~ according to claim 15.
100. (Withdrawn- Currently amended) An isolated nucleic acid whose sequence encodes a cellulase ~~cellulose~~ according to claim 19.
101. (Withdrawn- Currently amended) An isolated nucleic acid whose sequence encodes a cellulase ~~cellulose~~ according to claim 20.

102. (Withdrawn- Currently amended) An isolated nucleic acid whose sequence encodes a cellulase cellulose according to claim 21.
103. (Withdrawn) A recombinant expression vector comprising a nucleic acid sequence according to claim 96.
104. (Withdrawn) A recombinant expression vector comprising a nucleic acid sequence according to claim 97.
105. (Withdrawn) A recombinant expression vector comprising a nucleic acid sequence according to claim 98.
106. (Withdrawn) A recombinant expression vector comprising a nucleic acid sequence according to claim 99.
107. (Withdrawn) A recombinant expression vector comprising a nucleic acid sequence according to claim 100.
108. (Withdrawn) A recombinant expression vector comprising a nucleic acid sequence according to claim 101.
109. (Previously presented) The composition according to claim 6, wherein the fungus is a mutant strain of *Chrysosporium lucknowense* Garg 27K having accession number VKM F-3632D.
110. (Withdrawn- Currently amended) A method of saccharification of cellulose, comprising treating the cellulose with an isolated cellulase enzyme of claim 16 having at least 50% of its maximal cellulase activity at a neutral and/or alkaline pH.
111. (Withdrawn) The method of claim 110, wherein the cellulose is a lignocellulose biomass from agriculture, forest products, municipal solid waste, or other like sources.
112. (Currently amended) A composition comprising an isolated neutral and/or alkaline cellulase, said isolated neutral and/or alkaline cellulase is obtained from a wild type or mutant fungus of the genus *Chrysosporium*, wherein the fungus is

Chrysosporium lucknowense Garg 27K, accession number VKM F-3500D, *Chrysosporium pannorum*, *Chrysosporium keratinophilum*, *Chrysosporium lobatum*, *Chrysosporium merdarium*, *Chrysosporium queenslandicum*, or *Chrysosporium tropicum*, said composition has cellulase activity at a temperature between 40 °C and 60 °C, and at a pH of about 5 to about 12.

113. (New) A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least about 191 units of endo-1,4- β -glucanase activity per gram of dry composition, as measured by an endoviscometric assay, of an isolated cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*, wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D.
114. (New) A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least about 191 units of endo-1,4- β -glucanase activity per gram of dry composition, as measured by an endoviscometric assay, of an isolated cellulase obtained from a wild-type or mutant fungus of the genus *Chrysosporium*, wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D.